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Preliminary and Short Report

THIN-LAYER CHROMATOGRAPHY OF THE PIGMENTS ISOLATED FROM SOME TRICHOPHYTON SPECIES*

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As a preliminary step to our studies for the purpose of obtaining a complete identification of the pigment compounds produced by the dermatophytes, thin-layer chromatographic detection was made on the pigments isolated from the mycelia of *Trichophyton violaceum* and *Trichophyton rubrum*.

MATERIALS AND METHODS

A strain of *T. violaceum* (No. 1851) generously supplied from Dr. P. Pinetti of Cagliari University, Italy, a strain of *T. rubrum* obtained from the Fermentative Institute, Osaka, Japan, and also several strains of *T. rubrum* isolated from our own patients with trichophytosis were employed. They were grown on Sabouraud's dextrose-yeast extract agar (dextrose 4%, peptone 1% and yeast extract 1%) at 28° C. for about 4 weeks.

The mycelia were harvested, washed repeatedly with distilled water, extracted with acetone containing 0.1% hydrochloric acid, and the extract was concentrated to dryness. The residue was extracted with cyclohexane and then with diethylether to remove lipid materials. The remaining residue was dissolved in a small quantity of acetone and was subjected to thin-layer chromatography on silica gel (Wakogel B-5). For development, the following solvent systems were used:

System A, benzene-ethylmethylketone-formic acid—75:24:1 (v/v %).

System A′, benzene-ethylmethylketone-formic acid—69:30:1.

System A″, benzene-ethylmethylketone-formic acid—79:20:1.

System B, benzene-ethyl formate-formic acid—75:24:1.

The standard reference sample of Xanthomegnin used, isolated from *T. megnini*, was kindly given by Dr. G. Just of McGill University, Canada.

RESULTS

A schematic presentation of the thin-layer chromatograms of the crude pigment preparations which were obtained from the above-mentioned species is given in Fig. 1-A and 1-B, and compared with that of Xanthomegnin sample. The color tones of the spots as well as their fluorescence under ultraviolet light are indicated on the diagrams.

As is clear from the Fig. 1-A, the pigments from *T. violaceum* were resolved into 5 spots, each with yellow color, after development with the solvent system A. By means of solvent system B, 6 colored spots were detected as shown in Fig. 1-B. In addition to these, 4–6 spots apparently without color (not visible in daylight) but with notable fluorescence under ultraviolet light were obtained by each solvent system, though it remains to be determined whether they are of pigment nature or they are merely contaminants.

One of the colored spots was found to have always an Rf value identical with that of the authentic Xanthomegnin preparation,* even when the proportion of the developing solvent system was varied (the systems A, A′ and A″) or a separate system (system B) was employed. Therefore, it was tentatively identified as Xanthomegnin. This spot of Xanthomegnin changed its color tone from yellow to pinkish-red on standing for a few days at room temperature, and was easily distinguishable from the other spots. On the other hand, three yellow spots with an Rf lower than that of Xanthomegnin changed to faint orange on standing, while the similarly yellow-colored spots with a higher Rf than Xanthomegnin did not show such a change in color.

Furthermore, it is to be noted that, besides these spots, a deep reddish-brown color remained at the site of application and could not be removed with any of the several solvents used. Such a component does not seem to exist in the crude pigment preparation from *T. rubrum*, because in the chromatogram of the latter, only faint brown color was left at the site of application.

From the preparation of *T. rubrum* culture, 8 colored spots (visualized in daylight), accompanied by 5 fluorescent spots, were detected by a similar technic employing the system A (Fig. 1-A). With solvent system B, only 6 colored spots were obtained. In these cases, too, the presence of Xanthomegnin was clearly demonstrated. It is interesting to note that a distinct reddish-brown spot is present just above the site which is lacking in the chromatograms of the pigments from *T. vio-*

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* With this sample, a small fluorescent spot was detected in addition to the main colored spot. When solvent system B was used, two additional colored spots of minute size were also detected.

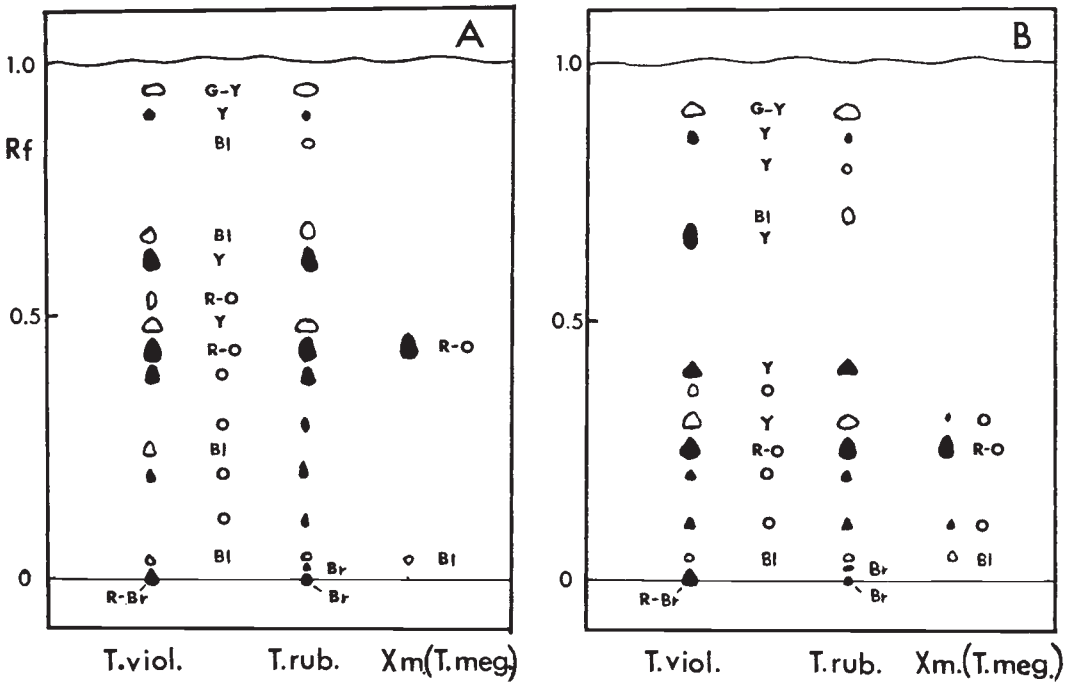

FIG. 1. Thin-layer chromatograms on silica gel of the crude pigment preparations from the mycelia of *Trichophyton violaceum* and *T. rubrum*. A: solvent system A, benzene-methylethylketone-formic acid (75:24:1), B: solvent system B, benzene-ethyl formate-formic acid (75:24:1). Developed for 25 minutes at room temperature. The color (indicated by black area) and fluorescence (blank area) at the time of 12 hours after development is indicated by abbreviation: Bl—blue; Br—brown; B—grey; O—orange; R—red; Y—yellow. T. viol.: *T. violaceum*. T. rub.: *T. rubrum*. Xm (T. meg.): Xanthomegnin from *T. megnini*.

laceum. The pigment preparations isolated from several other strains of the same species, *T. rubrum*, were examined with the same method with essentially the same results.

All the yellow spots on the chromatograms of the pigments from both species were found to become deep reddish-violet upon an addition of strong alkali, the result of being decolorized by a reduction with hydrosulfite and again restored to the original color upon auto-oxidation by atmospheric oxygen. These observations suggest strongly that these pigments are quinoid in nature.

DISCUSSION

Up to now, the existence of 3 to 4 kinds of pigments in a culture of individual species of the dermatophytes has been reported by various authors (1–4). However, the present work reveals that there are as many as 6 to 8 pigment compounds in the fungal mycelium.

It is interesting to note that, in spite of some differences as pointed out above, the thin-layer chromatograms of the pigment preparations from *T. violaceum* and *T. rubrum* showed generally rather notable resemblance; in either case, Xanthomegnin is present, and at least 4 yellow spots and 3 fluorescent spots in one chromatogram find their counter-parts in the other.

Xanthomegnin is a naphthoquinoid pigment, first isolated from *T. megnini* by Blank *et al.* (5), and is the only pigment of the dermatophytes for which the chemical structure has been elucidated. It was identified as 3,3′-bis[2-methoxy-5-hydroxy-7-(2-hydroxypropyl)-8-carboxyl-1,4-naphthoquinone lactone] by Just *et al.* (6). Its presence also in *T. rubrum* culture was found by Wirth (7). The present investigation has confirmed the existence of this pigment in the mycelia of *T. violaceum* as well as of *T. rubrum*. However, this pigment represents only one component of the 6 to 8 kinds of pigment compounds revealed by the thin-layer chromatography. The chemical nature of the other pigments is not yet clarified, though they were presumed to be also of quinoid nature. It is not improbable that they are anthraquinone derivatives, as evidenced by the finding of polyhydroxymethyl anthraquinone derivatives in *T. rubrum* as well as in *Microsporum cookei* reported by Koehne *et al.* (8).

It is now well established that the fungal cells of *T. violaceum* and *T. rubrum* contain several types of quinoid pigments. The physiological function of these pigments are completely unknown so

far. Because of the evidence indicating that they exist in an intracellular organelle similar in size and shape to mitochrondrion, their role as respiratory pigments is strongly suggested. Further studies are being carried out to elucidate this problem.

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